

A new species of the genus *Scutiger* (Anura: Megophryidae) from Medog of southeastern Tibet, China

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ABSTRACT

A new species of *Scutiger* Theobald, 1868 is described from Medog, southeastern Tibet, China, based on morphological and molecular data. The new species was previously identified as *Scutiger nyungchiensis*, but it can be differentiated from the latter and all other congeners by the following combination of characters: (1) medium adult body size, SVL 50.5–55.6 mm in males and 53.8–57.2 mm in females; (2) maxillary teeth absent; (3) web rudimentary between toes; (4) prominent, conical-shaped tubercles on dorsal and lateral surfaces of body and limbs; (5) tubercles covered by black spines in both sexes in breeding condition; (6) a pair of pectoral glands and a pair of axillary glands present and covered by black spines in males in breeding condition, width of axillary gland less than 50% of pectoral gland; (7) nuptial spines present on dorsal surface of first and second fingers, and inner side of third finger in males in breeding condition; (8) spines absent on the abdominal region; (9) vocal sac absent. In addition, the distribution and conservation status of the new species are also discussed.

Keywords: Eastern Himalayas; Tibet; *Scutiger nyungchiensis*; *Scutiger spinosus* sp. nov.; DNA barcoding

INTRODUCTION

The Tibet-Hengduan Mountain area is one of the 35 biodiversity hotspots of the world (Mittermeier, 2004) and supports an abundance of organisms, many of which are endemic to the region. Among the endemic fauna, the majority of species in the megophryid frog genus *Scutiger* Theobald, 1868 are known only from the montane habitats at high elevations between 1 900 m and 5 100 m in this region. Currently, there are 20

species recognized in the genus (Frost, 2015), of which six species are known from Tibet (Jiang et al., 2012), including *S. boulengeri* (Bedriaga, 1898), *S. maculatus* (Liu, 1950), *S. mammatus* (Güther, 1896), *S. nyungchiensis* Fei, 1977, *S. wuguanfui* Jiang et al., 2012, and *S. sikkimensis* (Blyth, 1854).

During our fieldwork from 2011 to 2013 in southeastern Tibet, we collected 16 specimens of megophryid frogs that were initially identified as *Scutiger nyungchiensis* from 62K, Medog (=Motuo). After morphological comparisons and genetic analysis with respect to topotype specimens of *S. nyungchiensis*, we were able to distinguish however, the *Scutiger* specimens from 62K, Medog can be readily distinguished from *S. nyungchiensis* by a suit of morphological characters and a significant genetic distance. Therefore, we describe the 62K population of *Scutiger* as a new species.

MATERIALS AND METHODS

From June 2011 to June 2013, a total of 16 individuals (12 males and four females) were collected from 62K, Medog, southeastern Tibet, China. Following euthanasia, liver tissues were taken from each individual and preserved in 95% ethanol, and all specimens were then fixed in 10% formalin solution and transferred to 75% ethanol after returning from the field. All specimens were designated as the type series, and

Received: 17 October 2015; Accepted: 20 December 2015

Foundation items: This study was supported by the "Light of West China" Program of the Chinese Academy of sciences (CAS), the Ministry of Science and Technology of China (2014FY210200, 2011FY120200), and the Animal Branch of the Germplasm Bank of Wild Species of Chinese Academy of Sciences (the Large Research Infrastructure Funding)

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DOI:10.13918/j.issn.2095-8137.2016.1.21

were deposited in Kunming Institute of Zoology, Chinese Academy of Sciences.

Morphological analysis

Morphological characters used and their measurement methods followed Fei et al. (2009). Measurements were taken with dial calipers and recorded to the nearest 0.1 mm.

The morphological characters and their abbreviations as: SVL, snout-vent length; HL, head length; HW, head width; SL, snout length; INS, internarial distance; IOS, interorbital distance; EHD, eye horizontal diameter; UEW, maximum width of upper eyelid; FAHL, forearm and hand length; FAW, maximum width of forearm; HAL, hand length; FML, femur (thigh) length; TBL, tibia (shank) length; TFL, length of tarsus and foot; FOL, foot length.

The new species was compared to museum specimens (Appendix) as well as descriptions in the literature: *Scutiger adun-genis* (Dubois, 1979), *S. bhutanensis* (Delorme & Dubois, 2001), *S. brevipes* (Liu, 1950), *S. nepalensis* (Dubois, 1973), and *S. chintingensis*, *S. glandulatus*, *S. gongshanensis*, *S. jiulongensis*,

S. liupanensis, *S. maculatus*, *S. muliensis*, *S. ningshanensis*, *S. pingwuensis*, *S. tuberculatus* and *S. wanglangensis* from Fei et al. (2009). The following museum abbreviations were used: CIB-Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China. KIZ-Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.

Molecular analysis

Total DNA was extracted from 15 specimens of seven Tibetan *Scutiger* species (Table 1), using a standard three-step phenol-chloroform method (Sambrook et al., 1989). A 561 base pair DNA fragment of the mitochondrial gene Cytochrome Oxidase Subunit I (COI) was amplified and sequenced for all samples using primers Chmf4 and Chmr4 (Che et al., 2012). Protocols for PCR and sequencing followed Che et al. (2012). All novel sequences were deposited in GenBank (KU243053-KU243067, Table 1). Sequences for three additional species of Tibetan *Scutiger* were downloaded from Genbank as well outgroup taxa including *Xenophrys*, *Brachytarsophrys* and *Leptolalax* (Table 1).

Table 1 Information of samples used in molecular analysis

Genus	Species	Locality	Specimen voucher No.	GenBank No.
<i>Scutiger</i>	<i>spinosus</i>	China: Medog, Tibet	KIZ011114 KIZ011100 KIZ012645	KU243053 KU243054 KU243055
	<i>nyingchiensis</i>	China: Nyingchi (=Linzhi), Tibet	KIZ017459 KIZ017460	KU243056 KU243057
	<i>sikimensis</i>	China: Yadong, Tibet	KIZ011127 KIZ07371	KU243058 KU243059
	<i>wuguanfui</i>	China: Medog, Tibet	KIZ011101 KIZ011102	KU243060 KU243061
	<i>gongshanensis</i>	China: Gongshan, Yunnan	CIB20070717001 CIB20070717002	KU243062 KU243063
	<i>boulengeri</i>	China: Bom (=Bomi), Tibet	KIZ06712 KIZ06713	KU243064 KU243065
	<i>jiulongensis</i>	China: Ganzi, Sichuan	KIZ045055 KIZ045056	KU243066 KU243067
	<i>boulengeri</i>	China: Gansu	— —	KJ082074 KJ082073
	<i>ningshanensis</i>	China: Shaanxi	— —	KF757393 KF757392
	<i>liupanensis</i>	China: Ningxia	— —	JN700835 JN700834
<i>Xenophrys</i>	<i>maosonensis</i>	—	—	KR087950
	<i>major</i>	—	—	KR087949
	<i>jingdongensis</i>	—	—	KR087947
<i>Leptolalax</i>	<i>minimus</i>	—	—	KR087759
<i>Brachytarsophrys</i>	<i>feae</i>	—	—	JN700843

All sequences were aligned and edited in MEGA 5 (Tamura et al., 2011). The best model of nucleotide substitution for each codon position was SYM+I, F81 and GTR+G determined using

jModeltest v1.0.1 (Posada, 1998). Bayesian inference (BI) was used to generate a phylogenetic relationship using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Using different model

for each codon position, the Markov chains were estimated for 10 million generations, and every 100 generations were sampled. The genetic distance between species was calculated using MEGA 5 with Kimura 2-parameter model (Che et al., 2012).

RESULTS

Morphological comparison

The results of the morphological comparisons between the *Scutiger* population from 62K, Medog and its congeners are summarized in Table 2. The morphological comparisons reveal that the *Scutiger* population at 62K, Medog can be readily distinguished from morphologically similar *S. nyngchiensis* and other congeners by a suite of diagnostic characters, including a light brown triangular pattern of pigmentation on the dorsal surface of the head, rudimentary webbing between toes, and prominent and conical shaped tubercles on the dorsal and lateral surfaces of the body and limbs.

Phylogenetic relationships

The 561 base pairs (bp) of COI sequences for 15 individuals were sequenced (Table 1). There are 155 and 202 potentially parsimony informative sites within genus *Scutiger* and between the ingroup and outgroup, respectively. The BI analysis supports the genus *Scutiger* as a monophyletic group. Five lineages are identified based on available data for *Scutiger* (Clade A-E, Figure 1). Species distributed in Tibet are recovered in Clade A, C, D, and E. The 62K population of *Scutiger*, along with *S. nyngchiensis* from Nyingchi, Tibet and *S. gongshanensis* from Gongshan, Yunnan, forms the lineage D. The 62K population of *Scutiger* differs from the phenotypically similar *S. nyngchiensis* and from *S. gongshanensis* by a genetic distance of 10.6% and 10.9% respectively. *Scutiger nyngchiensis* and *S. gongshanensis* are sister species, with a genetic distance of 8.5%.

Furthermore, the populations of *S. boulengeri* from Gansu and Tibet do not form a monophyletic lineage, and the genetic distance between the two populations is 8.1%.

Given that both morphological and molecular results support the *Scutiger* population from 62K, Medog as a distinct evolutionary lineage distinct from all other Tibetan *Scutiger*, therefore, following the species concept by and concordant evidence confirm species status (Wu & Murphy, 2015), herein we describe the 62K population of *Scutiger* as a new species.

Scutiger spinosus sp. nov. Jiang, Wang, Li and Che (Figures 2-4)

Synonyms

Scutiger nyngchiensis: Li et al., 2010; Jiang et al., 2012

Holotype: KIZ 011114 (Figure 2), an adult male from 62K, Medog, Tibet, China (N29°42'33.6", E95°34'56.0", elevation 2 705 m). Collected by Ke JIANG on 07 June 2011.

Paratypes: a total of 15 specimens (11 males and four females) from the same locality as holotype, including one adult male

(KIZ 011113) and two adult females (KIZ 011093, KIZ 011100), collected by Ke JIANG and Pi-Peng LI as same date as holotype; three adult males (KIZ 012645, 012647-48) and one adult female (KIZ 012646) collected by Ke JIANG, Kai WANG and Jiang XIE on 15 July 2012; and seven adult males (KIZ 013862-64, 013866-69) and one adult female (KIZ 013865), collected by Ke JIANG and Kai WANG on 17 June 2013.

Diagnosis: *Scutiger spinosus* sp. nov. is identified to the genus *Scutiger* by its molecular phylogenetic position and the following morphological characters: (1) maxillary teeth absent or indistinct; (2) vomerine teeth absent; (3) tympanum and tympanic ring entirely absent; (4) pupil vertical, elliptical; (5) pectoral and axillary gland present in males, and covered by black spines in breeding condition; (6) inner three fingers of males with black nuptial spines in breeding condition.

Scutiger spinosus sp. nov. is distinguished from all other congeners by the following combination of morphological characters: (1) medium adult body size, SVL 50.5-55.6 mm in males and 53.8-57.2 mm in females; (2) maxillary teeth absent; (3) web rudimentary between toes; (4) prominent, conical-shaped tubercles on dorsal and lateral surfaces of body and limbs; (5) tubercles covered by black spines in both sexes in breeding condition; (6) a pair of pectoral glands and a pair of axillary glands present and covered by black spines in males in breeding condition, width of axillary gland less than 50% of pectoral gland; (7) nuptial spines on dorsal surface of first and second fingers, and inner side of third finger in males in breeding condition; (8) spines absent on the abdominal region; and (9) vocal sac absent.

Description of the holotype: SVL 51.7 mm; body slightly compressed, moderately slender at waist; head large and flat, width nearly equal to length (HW/HL=1.04); snout rounded, slightly projecting beyond jaw; canthus rostralis obtuse, loreal region oblique and slightly concave; nostril midway between the tip of snout and eye; eye relatively large (EHD/HL=0.35); pupil vertical; interorbital space flat, interorbital distance less than upper eyelid width (IOS/UEW=0.90); tympanum and tympanic rim absent; a small tooth-like projection on anteromedial edge of mandible; jaws without teeth; tongue oval, slightly emarginate behind, papillae absent; choanae located against anterior border of palate, visible when viewed from below; vomerine teeth absent; vocal sac absent; supratympanic fold from posterior corner of orbit to shoulder, distinctly developed.

Forelimbs long; forearm enlarged; fingers slender, free of web or dermal fringes; relative length of fingers: I=II<IV<III; tips of fingers rounded, not dilated; subarticular tubercles absent; inner metacarpal tubercle almost equal to outer metacarpal tubercle, both large and flat; nuptial spines on dorsal surface of first and second fingers, and on inner side of third finger.

Hindlimbs moderately short, tibiotarsal articulation reaching the corner of mouth when adpressed, heels do not touch when folded at right angles to the body (FML/TBL=1.07); foot longer than shank (TFL/TBL=1.26); tips of toes round; dermal fringes

Table 2 Morphological comparisons of *Scutiger spinosus* sp. nov. and its congeners

Species	Male SVL n=12	Female SVL n=4	Maxillary tooth	teeth or Toes webbing	Spine patches on belly in males	Spine patches on breast, and the relative size	Nuptial spines on the number of fingers	Vocal sac
<i>spinosus</i>	50.5-55.6 n=12	53.8-57.2 n=4	Absent	Feeble	Absent	Two pairs, the inner one larger than the outer one	I, II, III	Absent
<i>adungensis</i>	71.0-73.0 n=2	—	Present	Feeble	Absent	One pair	I, II	Present
<i>bhutanensis</i>	53.0-53.4 n=2	—	Absent	Feeble	Absent	Two pairs, the inner one similar to the outer one	I, II	Absent
<i>boulengeri</i>	44.9-53.7 n=20	40.2-58.2 n=8	Absent or Present	Developed	Present	Two pairs, the inner one similar to the outer one	I, II, III	Absent
<i>brevipes</i>	68.0-80.0 n=10	58.0-68.0 n=9	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	I, II	Absent
<i>chintingensis</i>	42.0-50.3 n=22	48.0-52.8 n=6	Present	Developed	Absent	Two pairs, the inner one slightly larger than the outer one	I, II, III	Absent
<i>glandulosus</i>	68.0-90.0 n=17	58.0-83.7 n=14	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	I, II	Absent
<i>gongshanensis</i>	47.0-57.0 n=19	49.0-60.0 n=2	Present	Absent	Absent	One pair	I, II	Present
<i>jiulongensis</i>	67.4-81.5 n=20	—	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	I, II	Absent
<i>llupanensis</i>	40.6-48.0 n=20	52.0-59.5 n=3	Present	Feeble	Present	Two pairs, the inner one similar to the outer one	I, II, III	Absent
<i>maculatus</i>	65.4 n=1	69.0 n=1	Present	Developed	Absent	Two pairs, the inner one slightly larger than the outer one	I, II, III	Absent
<i>mammatus</i>	58.8-71.7 n=11	63.0-77.3 n=6	Absent or Present	Developed	Absent	One pair	I, II	Absent
<i>mullensis</i>	68.2-80.0 n=10	60.1-67.5 n=10	Absent	Feeble	Absent	One pair	I, II	Absent
<i>nepalensis</i>	68.0-73.5 n=4	59.5-64.5 n=2	Unknown	Unknown	Absent	Two pairs, the inner one similar to the outer one	I, II, III	Absent

Continued

Species	Male SVL	Female SVL	Maxillary tooth	teeth or Toes webbing	Spine patches on belly in males and the relative size	Nuptial spines on the number of fingers	Vocal sac
<i>ningshanensis</i>	51.0 <i>n</i> =1	41.0 <i>n</i> =1	Present	Feeble	Present	Two pairs, the inner one similar to the outer one	I , II , III
<i>nyingchiensis</i>	52.8-67.6 <i>n</i> =5	70.0 <i>n</i> =1	Present	Developed	Absent	Two pairs, the inner one slightly larger than the outer one	Absent
<i>pingwuensis</i>	60.7-75.8 <i>n</i> =20	77.5 <i>n</i> =1	Absent	Feeble	Present	Two pairs, the inner one much larger than the outer one	I , II , III
<i>sikimensis</i>	46.9-51.3 <i>n</i> =8	50.8-53.9 <i>n</i> =2	Present	Feeble	Absent	Two pairs, the inner one slightly larger than the outer one	Absent
<i>tuberculatus</i>	68.0-76.0 <i>n</i> =16	63.6-79.0 <i>n</i> =7	Absent	Feeble	Absent	Two pairs, the inner one much larger than the outer one	I , II
<i>wanglangensis</i>	52.7-58.2 <i>n</i> =6	64.3 <i>n</i> =1	Present	Feeble	Present	Two pairs, the inner one similar to the outer one	I , II , III
<i>wuguanfui</i>	77.5-83.8 <i>n</i> =6	107.4-116.7 <i>n</i> =2	Absent	Feeble	Absent	Two pairs, the inner one similar to the outer one	I , II , III
							Present

Information of *S. spinosus* sp. nov., *S. boulengeri*, *S. mammatus*, *S. nyingchiensis*, *S. sikimensis*, and *S. wuguanfui* are obtained by this study.

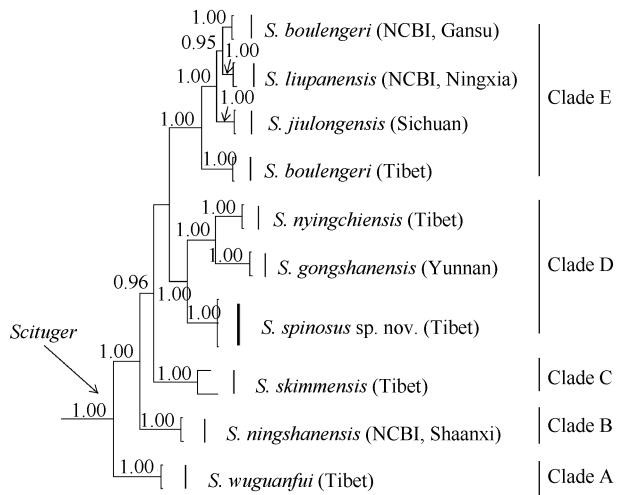


Figure 1 Bayesian inference tree based on barcoding COI data of the Tibetan congeners of the genus *Scutiger*

The numbers represent Bayesian posterior probabilities (only ≥ 90 were showed).



Figure 2 *Scutiger spinosus* sp. nov. holotype (KIZ 011114) in preservative (Photos by Ke JIANG)

A: Dorsolateral view; B: Dorsal view of body; C: Ventral view; D: Dorsolateral view of body, showing tubercles and spines; E: Dorsal view of hindlimbs, showing tubercles and spines on thigh; F: Ventral view of hand; G: Ventral view of foot.

feeble; relative length of toes: I<II<III<V<IV; subarticular tubercles absent; ridges on undersurfaces of toes absent; inner metatarsal tubercle elliptical and prominent, outer metatarsal tubercle absent.

Skin of dorsal side extremely rough. Forehead and upper lip relatively smooth, scattered small tubercles without spine; small



Figure 3 *Scutiger spinosus* sp. nov. paratype (KIZ 013867), adult male in life (Photos by Kai WANG)

A: Dorsolateral view; B: Dorsal view; C: Ventral view; D: Ventral view of hand; E: Ventral view of foot.



Figure 4 *Scutiger spinosus* sp. nov. paratype (KIZ 013865) adult female in life (Photos by Kai WANG)

A: Dorsolateral view; B: Ventral view; C: Ventral view of hand; D: Ventral view of foot.

tubercles present below supratympanic fold, each tubercle bearing one black spine; supratympanic fold, top of head, dorsal sides of body and limbs, and flanks covered with large, prominent, conical-shaped tubercles, all bearing black spines except on dorsal side of forearm; small tubercles scattered among the larger tubercles, mostly bearing black spine. Skin on ventral surface of body mostly smooth; numerous small black spines present on margin of lower jaw, small patches of black spines present near armpit. A pair of pectoral glands and a pair of axillary glands present on chest, pectoral twice longer than axillary, both covered by dense black spines.

Coloration in life: A light brown, triangular pattern of pig-

mentation is observed on the dorsal surface of the anterior portion of the head, the apex is at the tip of snout and the base is between eyes. Several dark spots overlay the triangular pattern. Lateral surfaces of the head are dark brown; upper lip is creamy white, with irregular shaped brown mottling. The coloration of the remaining dorsal surfaces of the head, body, lower forelimbs, and hind limbs are dark brownish gray. The flanks are light brown and gradually fade into light brownish yellow ventrally. Dorsal surfaces of the upper forelimbs are light brownish-yellow, with few transverse stripes of darker brown; dorsal surfaces of the lower forelimbs are dark gray, with few

light gray transverse bands. Light mottling patterns of pigmentation are also observed on the dorsal surfaces of the hind limbs. Ventral surfaces of the head, body, and limbs are uniform light yellow.

The coloration in preservative closely resembles the coloration in life, except that the off-white coloration of the lips and the light brownish yellow of the venter fade and become light gray.

Variation: Measurements of type series are shown in Table 3. Individuals of type series are generally uniform in external characters, except for the characters related to sexual dimorphism in the males.

Table 3 Morphological measurements (mm) of the type series of *Scutiger spinosus* sp. nov.

Number	Status	Sex	SVL	HL	HW	SL	INS	IOS	UEW
KIZ011114	Holotype	Male	51.7	18.7	19.4	7.5	4.3	4.5	5.0
KIZ013868	Paratype	Male	53.6	18.4	18.4	6.8	4.0	3.8	5.1
KIZ013866	Paratype	Male	55.3	18.0	18.9	7.0	4.3	3.8	5.1
KIZ013863	Paratype	Male	54.4	17.7	18.6	6.6	4.4	3.8	5.0
KIZ013867	Paratype	Male	51.8	18.3	18.2	6.9	4.2	3.6	4.8
KIZ013862	Paratype	Male	54.3	18.2	17.8	6.7	4.6	4.1	4.7
KIZ013869	Paratype	Male	54.5	18.0	18.2	6.1	4.4	4.5	5.2
KIZ013864	Paratype	Male	52.1	17.7	17.3	6.9	4.3	3.7	5.1
KIZ011113	Paratype	Male	50.5	18.0	18.0	7.3	4.1	4.4	4.4
KIZ011099	Paratype	Male	55.5	19.1	19.8	7.9	4.6	4.6	4.7
KIZ012645	Paratype	Male	52.7	19.3	19.8	7.0	4.2	4.4	4.7
KIZ012648	Paratype	Male	55.3	18.8	18.9	7.2	4.2	3.7	5.1
KIZ012647	Paratype	Male	55.6	19.8	19.8	7.3	4.5	4.1	5.4
	Range		50.5-55.6	17.7-19.8	17.3-19.8	6.1-7.9	4.0-4.6	3.6-4.6	4.4-5.4
	Average		53.6	18.5	18.7	7.0	4.3	4.1	4.9
	Ratio to SVL (%)			34.5	34.9	13.1	8.0	7.6	9.1
KIZ013865	Paratype	Female	54.2	17.4	17.9	6.3	4.5	3.5	4.7
KIZ011093	Paratype	Female	57.2	19.3	19.5	7.5	4.8	4.4	5.8
KIZ011100	Paratype	Female	56.9	18.3	18.4	7.1	4.6	4.1	5.0
KIZ012646	Paratype	Female	53.8	18.4	18.6	7.0	4.7	4.4	5.5
	Range		53.8-57.2	17.4-19.3	17.9-19.5	3.9-7.5	4.5-4.8	3.5-4.4	4.7-5.8
	Average		55.5	18.4	18.6	6.4	4.7	4.1	5.3
	Ratio to SVL (%)			33.2	33.5	11.5	8.5	7.4	9.5
Number	EHD	FAHL	FAW	HAL	FML	TBL	TFL	FOL	
KIZ011114	6.5	26.6	6.9	14.2	22.3	20.9	37.4	26.4	
KIZ013868	6.4	27.2	7.3	13.4	23.6	21.6	37.3	25.2	
KIZ013866	6.6	29.4	6.8	13.3	23.6	21.6	39.0	26.7	
KIZ013863	6.8	29.3	7.0	14.2	23.3	21.6	36.1	25.4	
KIZ013867	6.4	27.9	6.5	13.9	22.5	20.6	35.2	24.6	
KIZ013862	6.6	28.6	6.5	14.1	22.8	22.3	34.7	26.5	
KIZ013869	6.9	28.6	7.7	13.5	23.0	21.4	37.7	26.3	
KIZ013864	6.2	27.3	6.9	14.1	21.1	21.3	37.5	26.5	
KIZ011113	6.4	25.5	5.5	13.6	22.7	20.9	36.6	24.2	
KIZ011099	6.6	30.1	6.9	16.1	26.6	24.3	42.0	29.2	
KIZ012645	6.0	27.6	6.0	13.2	23.0	20.9	37.7	25.8	

Continued

Number	EHD	FAHL	FAW	HAL	FML	TBL	TFL	FOL
KIZ012648	6.5	25.6	6.5	14.4	23.5	21.0	38.0	27.1
KIZ012647	6.3	26.1	6.7	14.1	23.2	21.7	26.3	25.3
Range	6.0-6.8	25.5-30.1	5.5-7.7	13.2-16.1	22.3-26.6	20.6-24.3	26.3-42.0	24.2-29.2
Average	6.5	27.7	6.7	14.0	23.2	21.5	36.6	26.1
Ratio to SVL (%)	12.1	51.7	12.5	26.1	43.3	40.1	68.3	48.7
KIZ013865	6.5	26.4	3.9	13.5	19.6	19.6	32.0	21.6
KIZ011093	6.3	29.1	5.2	15.3	22.8	20.9	37.3	24.2
KIZ011100	6.4	28.5	4.6	15.8	23.3	20.7	37.9	26.3
KIZ012646	7.0	27.4	4.0	15.0	21.7	19.9	34.5	24.0
Range	6.3-7.0	26.4-29.1	3.9-5.2	13.5-15.8	19.6-23.3	19.6-20.9	32.0-37.9	21.6-26.3
Average	6.6	27.9	4.4	14.9	21.9	20.3	35.4	24.0
Ratio to SVL (%)	11.9	50.3	7.9	26.8	39.5	36.6	63.8	43.2

Abbreviations: SVL: snout-vent length; HL: head length; HW: head width; SL: snout length; INS: internarial distance; IOS: interorbital distance; UEW: width of upper eyelid; EHD, eye horizontal diameter; FAHL, forearm and hand length; FAW, width of forearm; HAL, hand length; FML, femur length; TBL, tibia length; TFL, length of tarsus and foot; FOL, foot length.

Sexual dimorphism: In males, small patches of black spines are present on the armpit, and a pair of pectoral glands and a pair of axillary glands are present on the chest, with the former ones much larger than the latter ones. Both pectoral and axillary glands are covered by black spines in breeding condition. Nuptial black spines are present on the dorsal surfaces of the first and second finger and the inner side of the third finger with in breeding condition. Forearms of males are much stronger than females (FAW male/female=1.5). In breeding condition, spines on the dorsal and lateral surfaces of males are more distinct and denser than females. Vocal sac and lineae masculinae are absent in males.

Ecological notes: The new species inhabits the mixed forest at high elevation, sympatric with *Scutiger wuguanfui*, *Nanorana medogensis* (Fei and Ye, 1999), and *Liurana xizangensis* (Hu, 1977). Breeding pairs were found under logs during the day and in the streams and ponds at night from the beginning to the end of June. The breeding habitats include slow-flowing streams and small to medium permanent ponds, with brownish, slightly acidic water. Males display inguinal amplexus, where the males grasp the females at the waist (Figure 5). Eggs are laid in donut-shaped masses and are attached to the under surfaces of logs and rocks (Figure 6). There is no evidence of

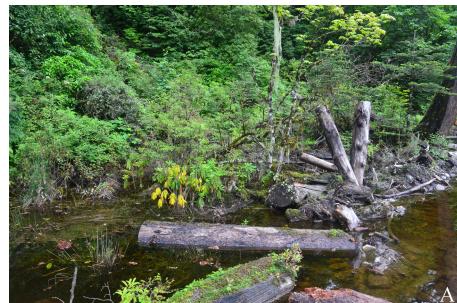


Figure 6 Breeding habitat (A) and eggs (B) of *Scutiger spinosus* sp. nov. at 62K of Medog, southeastern Tibet, China (Photos by Kai WANG and Pi-Peng LI)



Figure 5 Breeding pair of the *Scutiger spinosus* sp. nov., showing the inguinal amplexus (Photos by Kai WANG)

A: lateral view; B: ventral view.

nuptial spines on the fingers and pectoral and axillary glands in mid July. Tadpoles overwinter and take more than one year to develop into metamorphic juveniles, which is a common adaptation for high-elevation amphibians.

Etymology: The specific epithet *spinosus*, is derived from the Latin word *spina*, meaning spine and combined with *ōsus* to form an adjective indicating an abundance of the noun, *spina*. *Spinosus* is in reference to the conical-shaped tubercles and the numerous spines on the dorsal surfaces of this species.

Based on the Latin name we suggest the English common name to be “Spiny Lazy Toad”, the Chinese name as “Ci You Chi Tu Chan” (刺疣齿突蟾).

Morphological Comparisons: *Scutiger spinosus* sp. nov. is morphologically most similar to *S. nyngchiensis* and *S. boulengeri*, in which both species have similar body size, brownish gray dorsal coloration, and distinct tubercles on the dorsal surface of the body. However, the new species can be distinguished readily from *S. nyngchiensis* (Figure 7) by having rudimentary webbings on toes (v.s. well developed), prominent, conical-shaped tubercles on the dorsal and lateral surfaces of the body and limbs (v.s. tubercles elongated, relatively low, and not conical in shape), as well as by the presence of a distinct, light brown, triangular pattern on the dorsal surface of the head (v.s. absent), presence of large tubercles on the dorsal surfaces of thigh (v.s. absent), presence of spines on the upper arm (v.s. absent), presence of numerous, spine-bearing, small tubercles scattered among the large tubercles on the dorsal and lateral surfaces of the body and limbs (v.s. absent), and the presence of spines on the tubercles on the dorsal surface of the body and limbs in females (v.s. absent); and from *S. boulengeri* by having rudimentary webbings on toes (v.s. well developed), prominent, conical shaped, spine-bearing tubercles on the dorsal and lateral surfaces of the body (v.s. relatively flat, not conical shaped, with smaller spines), and by the absence of spines on the abdominal region in breeding condition in males (v.s. present).



Figure 7 Male and female of *Scutiger nyngchiensis* (Photos by Da-Hu ZOU, Bao-Lin ZHANG and Ke JIANG).

Dorsolateral view (A), ventral view (B) and ventral view of foot (C) in life, and dorsal view in preservative (D) of the adult topotypic male (KIZ 017460) from Bujiu, Nyingchi, Tibet, China. Dorsolateral view in life (E) and in preservative (F) of the adult female (KIZ 016237), showing the absence of spines on the dorsal surface of the body, from Gongbo'gyamda (=Gongbu'jiangda), Tibet, China.

Scutiger spinosus sp. nov. is closely related to *S. gongshanensis* based on the molecular phylogeny, but the new species differs from the latter by the absence of vocal sacs in males (v.s. presence), presence of axillary glands (v.s. absent) and presence of black spines on dorsal tubercles (v.s. absent). Additionally, the new species differs from the sympatric *S. wuguanfui*, by having a distinct dorsal coloration (brownish gray v.s. dark purplish brown), a smaller adult body size (SVL 50.5-55.6 mm in males, 53.8-57.2 mm in females v.s. 77.5-83.8 mm in males, 107.4-116.7 mm in females), prominent, conical, spine-bearing tubercles on the dorsal and lateral surfaces of body and limbs (v.s. flat protuberances that are relatively smooth), as well as by the absence of vocal sacs in males (v.s. presence).

DISCUSSION

Our molecular data shows that all known *Scutiger* species form a monophyletic group (Figure 1). Surprisingly, despite the closer geographic proximity and similar external morphology between *S. nyngchiensis* and *S. spinosus*, *S. nyngchiensis* is more distantly related to *S. spinosus* than *S. gongshanensis*. *Scutiger gongshanensis* is distributed much farther away in the Hengduan Mountain Range on the east and has quite different external morphology. Additionally, *Scutiger boulengeri* is paraphyletic based on our molecular phylogeny, which might be the results of mitochondrial gene introgression with other sympatric *Scutiger* species (Chen et al., 2009), existence of cryptic species, or mis-identification of congeners. Further phylogenetic and phylogeographic studies are needed to gain a better understanding of this interesting relationship.

Recently, Sarania et al. (2015) reported *S. nyngchiensis* from the China-India Disputed Region based on photographic evidence without voucher specimens. However, based on the morphological characters described and shown in their photos, such as the rudimentary webbings on toes, the presence of spines on tubercles in females, and an inverted triangular mark of pigmentation from the upper eyelid to the snout, these individuals possess diagnostic characters of the new species, and thus are likely to be *S. spinosus* instead of *S. nyngchiensis*. Further examinations of voucher specimens from the region are needed to ensure the taxonomic status of this population.

Although the new species is locally abundant, its pristine habitats are currently under great threats from rapid developments of roads in southeastern Tibet. Several breeding pools of the new species were eliminated entirely during the sampling period of our study, and significant deforestations were observed along the roads. Because the new species and other amphibians from the same habitats, namely *S. wuguanfui*, *Nanorana medogensis* and *Litorana xizangensis* are believed to be restricted to the mixed forests in this region, such deforestation and destructions of breeding habitat could be devastating to the long-term survival of these endemic amphibians. We recommend immediate attention to the conservation and protection of habitats in this area including the type locality of the new species. Given the recent human incursion into these areas, in-depth assessments of the ecology and population

status of the endemic amphibians including the new species *Scutiger spinosus* are needed now more than ever.

ACKNOWLEDGEMENTS

We thank to Mr. Bao-Lin ZHANG (KIZ) and Mr. Jiang XIE (North China Electric Power University Science and Technology College), and the volunteers, Mr. Tao LIANG, Mr. Duan YOU, Mr. Ya-Di HUANG and Mr. Ya-Qiang SUN, who helped with fieldwork in Tibet, Dr. Yun-Ke WU (Cornell University) and Dr. Yu ZENG (University of California, Berkeley) for providing some literatures.

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APPENDIX

The following specimens were examined:

- Scutiger boulengeri* (*n*=28): KIZ 012573, KIZ 012581, KIZ 012583-84 (4♂♂), Nyalam (=Nielamu), Tibet; KIZ 012605 (1♂), KIZ 012602-04 (3♀♀), Tingri (=Dingri), Tibet; KIZ 014935-39, KIZ 014941-42, KIZ 014944-46 (9♂♂), KIZ 014948, KIZ 014950 (2♀♀), Qamdo (=Changdu), Tibet; KIZ 012606-07, KIZ 012609-11, KIZ 012614 (6♂♂), KIZ 012616-18 (3♀♀), Ndong (=Naidong), Tibet.
- Scutiger glandulatus* (*n*=7, unmeasured): KIZ 016477-80, KIZ 016482 (5♂♂), KIZ 016481, KIZ 016483 (2♀♀), Ganzi, Sichuan.

- Scutiger mammatus* (*n*=17): KIZ 016469-70, KIZ 017293-95, KIZ 017447-48, KIZ 017454-56, KIZ 017458 (11♂♂), KIZ 016471, KIZ 017288-89, KIZ 017449, KIZ 017457 (5♀♀), Jomda (=Jiangda), Tibet; KIZ 014035 (1♀), Baxoi (=Basu), Tibet.
- Scutiger nytingchiensis* (*n*=6): KIZ 017459-60 (2♂♂, Nyingchi); KIZ 09416, KIZ 019415-16 (3♂♂), KIZ 016217 (1♀), Gongbo'gyamda (=Gongbu'jiangda), Tibet.
- Scutiger sikimensis* (*n*=10): KIZ 011127, KIZ 013983-88, KIZ 013990 (8♂♂), KIZ 013982, KIZ 013989 (2♀♀), Yadong, Tibet.
- Scutiger wuguanfui* (*n*=8): KIZ 01101-02, KIZ 011109-11, KIZ 012649 (6♂♂), KIZ 011116, KIZ 012650 (2♀♀), Medog, Tibet.